REMARKS

I. Status of Claims.

Claims 1-11 are presently pending. Claims 1-11 stand rejected.

II. Amendment to the claims.

Claims 6 has been amended. Claims 6-11 have been cancelled. No new matter is believed to be introduced by these amendments.

III. Interview.

An interview concerning the instant application was conducted on March 26, 2003 with Examiner Meller of the United States Patent Office and Applicants' attorney John C. Serio. Applicants thank Examiner Meller for courtesies extended in the granting and conducting of the interview. The Examiner suggested, during the interview, that 35 USC 112 rejections may be addressed by providing a reference that more clearly defines the use of the word effectors. Applicants subsequently provided Examiner Meller with a text book chapter concerning the world "effectors" as used by those skilled in the art.

Applicants had a supplemental interview with the Examiner related to the submitted reference. During the supplemental interview no agreement was reached regarding the use of the word effectors, however, the Examiner indicated that he would further consideration Applicants' claimed subject matter in light of the submitted reference.

Applicants had a further interview with the Examiner regarding issues raised within the advisory action. As a result of this interview no agreements were reached, however, Applicants have cancelled claims 6-11 and have attached to this response (Appendix A), as a convenient

reference a text book chapter previously submitted related to the use of the word "effectors" as used within the art. Applicants thank the Examiner for the opportunity to discuss the pending application.

IV. Rejection of claims 7-11 under 35 USC 112, first paragraph.

In the Office Action dated January 27, 2003 the Examiner rejected newly added claims 7-11 under 35 USC 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and/or use the invention. The Examiner stated that the phrase, " a compound having a means for modulating enzymatic activity of DPIV and DPIV analogous enzymes' is not supported by the instant specification.

In the interest of moving the application to allowance Applicants have cancelled claims 6-11 without prejudice.

II. Claims 1-11 rejected under 35 USC 112, first paragraph.

The Examiner further rejected claims 1-11 under 35 USC 112, first paragraph because the specification, while being enabling for a method of administering to a mammal a therapeutically effective amount of an inhibitor of DPIV does not reasonably provide enablement for administering any and all effectors for reducing enzymatic activity of DPIV and DPIV analogous enzymes. Claims 6-11 have been cancelled as noted above. Applicants respectfully traverse this rejection in regard to claims 1-5.

The use of the word effectors is described within the specification to express an activity upon DP IV and DP IV analogous enzymatic activity that is not restricted to the inhibition of

enzymatic activity but more clearly that of an effect upon these enzymes that results in the influence of their activity. In particular, in paragraph 47 of the instant specification the effectors are described not only as inhibitors, but also "substrates, pseudosubstrates, inhibitors of DP IV expression, binding proteins or antibodies to those enzyme proteins or combinations of those different substances that reduce the DP IV or DP IV analogous protein concentration in the mammalian organism." (Applicants' specification paragraph 47). Applicants' specification sets forth other methods that can influence the activity of enzymatic activity of DPIV. Applicants would respectfully request that this rejection be withdrawn.

V. Rejection of claims 1-5 and 7-11 under 35 USC 112, second paragraph.

The Examiner further rejected claims 1-5 and 7-11 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention. Specifically, the Examiner objected to the phrase "an effector for reducing enzymatic activity of DPIV and DPIV analogous enzymes" as being confusing and therefore failing to clearly point out Applicants' invention. Applicants respectfully traverse the rejection of claims 1-5 under section 112 second paragraph.

Applicants have attached to this response a chapter from a textbook well known and respected in the art (Appendix A), which was submitted to the Examiner during the course of the above noted telephonic interview. It is clear that the word "effector" is a term known by those skilled in the art. The term "effector", as known by those skilled in the art, describes molecules that bind to enzymes that can either increase or decrease their activities. (Molecular Cell Biology 2nd Edition, Darnell, Lodish and Baltimore page 63).

Within the specification the word "effectors" is used in at least paragraphs 2, 12, 13, 43, 44, 47 and 50. Therein "effectors" describe the reduction of DP IV and DP IV analogous enzymatic activity. Nowhere is it required that the reduction be complete inhibition of enzymatic activity but rather, reduction may be something less than complete inhibition.

For example, paragraph 47 of the instant specification describes effectors not only as inhibitors, but also as "substrates, pseudo substrates, inhibitors of DP IV expression, binding proteins or antibodies to those enzyme proteins or combinations of those different substances that reduce." It is respectfully submitted that the term "effectors" is one that is well known, definitive and understood by those skilled in the art. Under Section 112, second paragraph claims are to be construed as by those of ordinary skill in the art *Schenck v. Nortron Corp.*, 713 F.2d 782, 7886087, 218 USPQ 698, 701-02 (Fed. Cir. 1983) (emphasis added). Applicants have clearly defined their claim invention so that is clearly understood by those skilled in the art as required by the provision of 35 USC 112, second paragraph and respectfully request that the rejection of claims 1-5 be withdrawn.

The Examiner further rejected claims 7-11 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention. In the interest of moving the present applicant to allowance, Applicants have cancelled claims 6-11 without prejudice

VI. Rejection of Claims 1-11 under Double Patenting.

The Examiner rejected claims 1-11 under the judicially created doctrine of double patenting over claims 1-4 of U.S. Patent No. 6,319,893. As noted above Applicants have

cancelled claims 6-11. Applicants upon notification of allowable subject matter may execute an acceptable terminal disclaimer.

VII. Rejection of Claim 6 under 35 USC 102(b).

The Examiner rejected claim 6 under 35 USC 102 (b) as being anticipated by WO 97/40832. The Examiner states that Applicants have not qualified the patient as having hypoglycemia. In the interest of moving the instant application to allowance, Applicants have cancelled claim 6. Applicants respectfully request that this rejection be withdrawn.

CONCLUSION

For the foregoing reasons and with the above requested amendments, Applicants believe this application is in condition for allowance which is respectfully requested.

Respectfully submitted,

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Dated June 26, 2003

Appendix A

Molecular Cell Biology

SECOND EDITION

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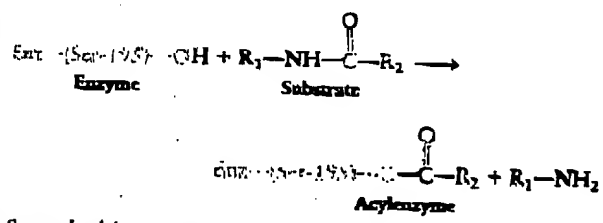
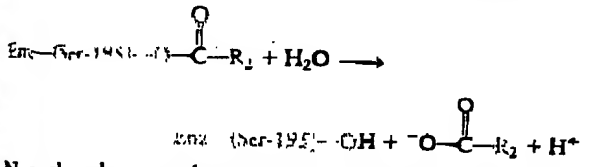
The hydrolysis reaction proceeds in two main steps. First, the peptide bond is broken and the carboxyl group is transferred to the hydroxyl residue of serine 195:



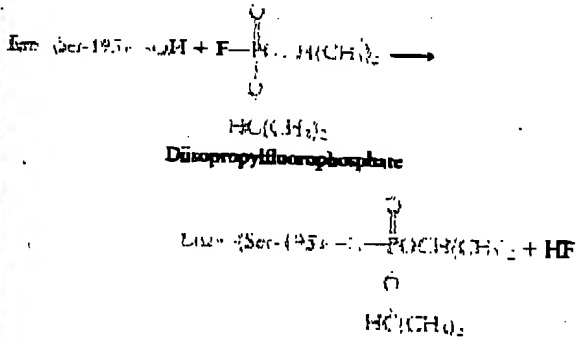
Second, this *acylenzyme* intermediate is hydrolyzed:



Note that the second step restores the enzyme to its original state.

Aspartate 102 and histidine 57 facilitate the acylation reaction by removing the proton from serine 195 and adding it to the nitrogen of the departing amino group (Figure 2-20). In a similar manner, aspartate 102 and histidine 57 facilitate the hydrolysis of the acylenzyme. These enzymatically catalyzed steps—transfer of a proton from the enzyme to the substrate, formation of a covalent acylserine intermediate, and hydrolysis of the acylenzyme—all drastically reduce the overall activation energy of the proteolysis reaction.

The hydroxyl group on serine 195 is unusually reactive. The concept of an "active" serine residue at the active site predated the determination of the crystal structure of chymotrypsin. It was already known, for example, that the compound diisopropylfluorophosphate is a potent inhibitor of chymotrypsin; it reacts only with the hydroxyl on serine 195 to form a stable covalent compound that irreversibly inactivates the enzyme:

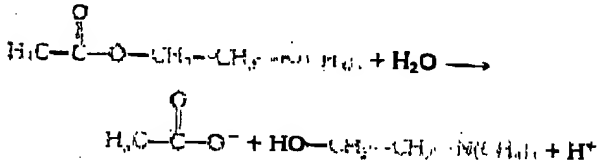


Trypsin and Chymotrypsin Have Different Substrate-binding Sites A comparison of trypsin and chymotrypsin will emphasize the nature of the specificity of enzymatically catalyzed reactions. About 40 percent of the amino acids in these two molecules are the same; in particular, the amino acid sequences in the vicinity of the key serine residue are identical:

195
—Gly—Asp—Ser—Gly—Gly—Pro

The three-dimensional structures and catalytic mechanisms of these two enzymes are also quite similar, indicating that they evolved from a common polypeptide. The major difference between trypsin and chymotrypsin is found in the side chains of the amino acids that line the substrate-binding site. The negatively charged amino acids in this area of the trypsin molecule facilitate the binding of only positively charged (lysine or arginine) residues, instead of hydrophobic ones.

Other Hydrolytic Enzymes Contain Active Serine Other, mostly unrelated, hydrolytic enzymes also contain an active serine residue that is essential for catalysis. For example, acetylcholinesterase catalyzes the hydrolysis of the neurotransmitter acetylcholine to acetate and choline:

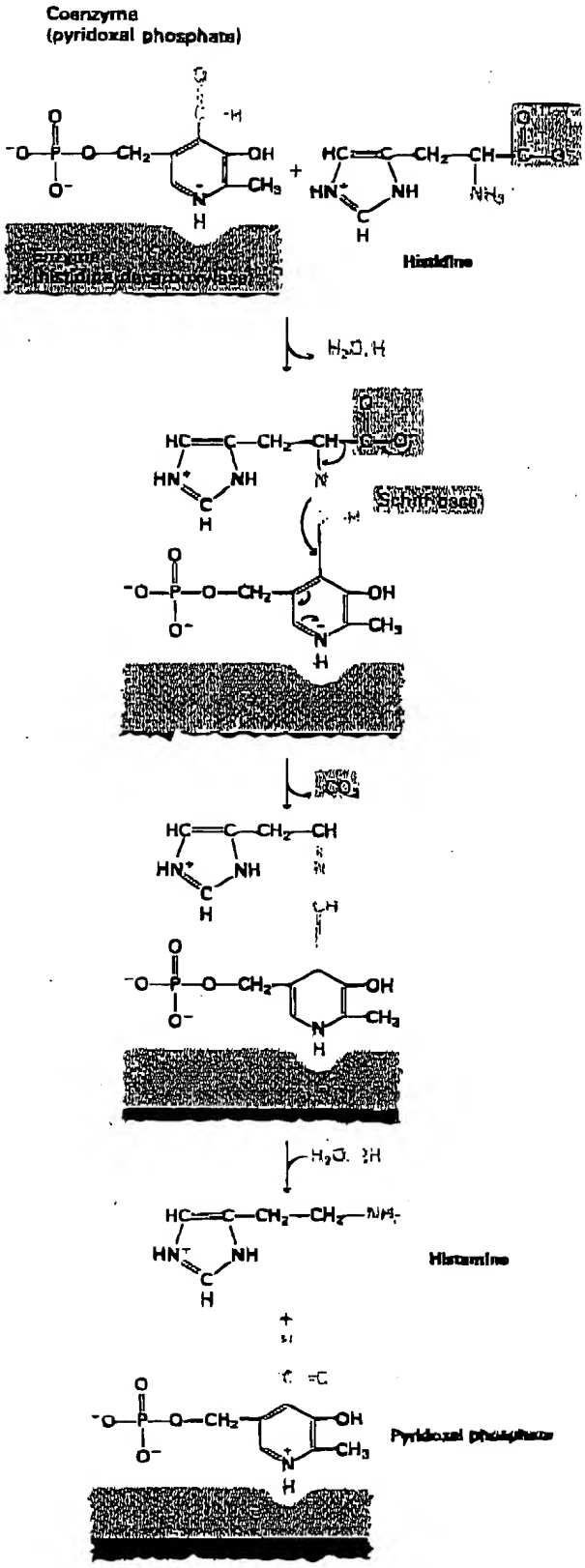


Diisopropylfluorophosphate is a potent, irreversible inhibitor of acetylcholinesterase as well as of chymotrypsin. The compound is lethal to animals because it blocks nerve transmission by causing a buildup of the transmitter substance. (The action of this transmitter is discussed in Chapter 20.)

Coenzymes Are Essential for Certain Enzymatically Catalyzed Reactions

Many enzymes contain a *coenzyme*—a tightly bound small molecule or prosthetic group essential to enzymatic activity. Vitamins required in trace amounts in the diet are often converted to coenzymes. Coenzyme A, for instance, is derived from the vitamin pantothenic acid; the coenzyme pyridoxal phosphate is derived from vitamin B_6. To cite just one example of how coenzymes function, we consider pyridoxal phosphate. The aldehyde group

can form a covalent complex called a *Schiff base* with an $—NH_2$ group of an amino acid, which facilitates or lowers the activation energy for the breaking of bonds to the carbon of the amino acid. Figure 2-21 shows how pyridoxal phosphate catalyzes the decarboxylation of histidine to form histamine—a potent dilator of small blood vessels. Histamine is released by certain cells in the course of allergenic hypersensitivity.



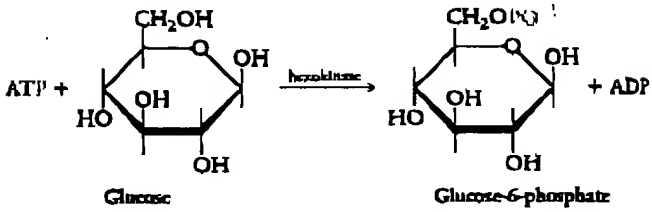
◀ **Figure 2-21** Pyridoxal phosphate, a coenzyme, participates in many reactions involving amino acids. When it is bound to histidine decarboxylase, as in this example, it forms a Schiff base with the α amino group of histidine. The positive charge on the nitrogen of pyridoxal phosphate then attracts the electrons from the carboxylate group of the histidine, via a charge relay system. This weakens the bond between the α carbon of the histidine and the carboxylate group, causing the release of CO_2. Finally, histamine, the reaction product, is hydrolyzed from the pyridoxal complex.

Substrate Binding May Induce a Conformational Change in the Enzyme

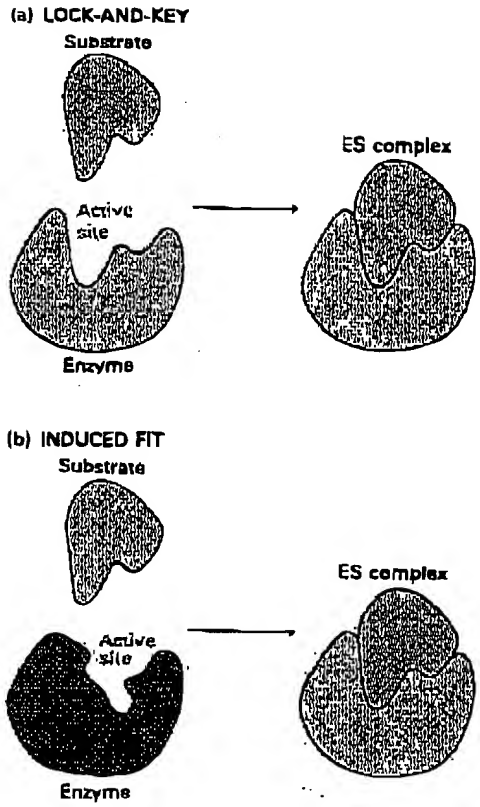
When a substrate binds to an enzyme, molecules of complementary charge or shape, or both, may simply fit together into a complex stabilized by a variety of noncovalent bonds. Such an interaction resembles the fitting of a key into a lock and is said to occur by a *lock-and-key* mechanism (Figure 2-22a).

In some enzymes, the binding of the substrate induces a conformational change in the enzyme that causes the catalytic residues to become positioned correctly. Molecules that attach to the substrate-binding site, or *recognition site*, of the enzyme but that do not induce a conformational change are not substrates of that enzyme. Thus an enzyme differentiates between a substrate and a nonsubstrate in two ways: Does the potential substrate bind to the enzyme? If so, does it induce the correct conformational change? When both criteria are met, the enzyme-substrate complex is said to demonstrate *induced fit* (Figure 2-22b).

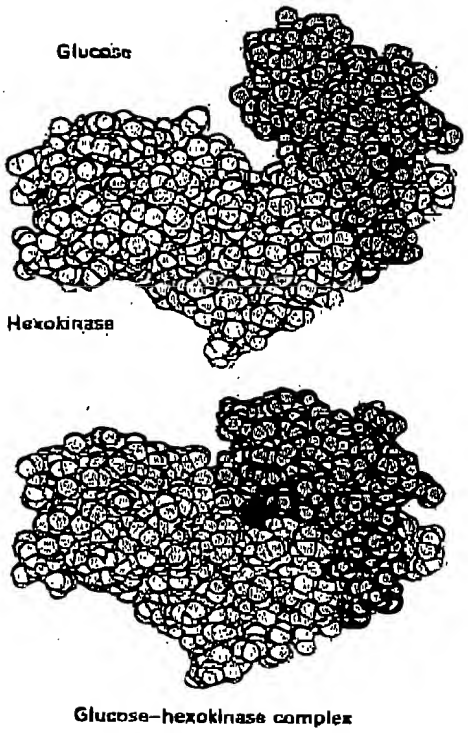
An important example of induced fit is provided by the enzyme hexokinase, which catalyzes the transfer of a phosphate residue from ATP to a specific carbon atom of glucose:



This is the first step in the degradation of glucose by cells. X-ray crystallography has shown that hexokinase consists of two domains. The binding of glucose induces a major conformational change that brings these domains closer together and creates a functional catalytic site (Figure 2-23). Only glucose and closely related molecules can induce this conformational change, ensuring that the enzyme is used to phosphorylate only the correct substrates. Molecules such as glycerol, ribose, and even water may bind to the enzyme at the recognition site but cannot induce the requisite conformational change, so they are not substrates for the enzyme.



▲ **Figure 2-22** Two mechanisms for the interaction of an enzyme and a substrate. (a) In the lock-and-key mechanism, the substrate fits directly into the binding site of the enzyme. (b) If binding occurs by induced fit, the substrate induces a conformational change in the enzyme that appropriately positions the substrate for catalysis.



▲ **Figure 2-23** The conformation of hexokinase changes markedly when it binds the substrate glucose: the two domains of the enzyme come closer together to surround the substrate. Molecules such as the five-carbon sugar ribose can also bind to hexokinase by forming specific hydrogen bonds with groups in the substrate-binding pocket of the enzyme, but only glucose can form all of the bonds that cause the enzyme to change its conformation. *Courtesy of Dr. Thomas A. Steitz.*

The Catalytic Activity of an Enzyme Can Be Characterized by a Few Numbers

Enzymatic specificity is usually quantified by discrimination ratios: a good substrate may be cleaved 10,000 times as fast as a poor substrate. The catalytic power of an enzyme on a given substrate involves two numbers: K_m, which measures the affinity of the enzyme for its substrate, and V_{max}, which measures the maximal velocity of enzymatic catalysis. Equations for K_m and V_{max} are most easily derived by considering the simple reaction

$$S \longrightarrow P$$
$$(\text{substrate} \longrightarrow \text{product})$$

in which the rate of product formation depends on [S], the concentration of the substrate, and on [E], the concentration of the catalytic enzyme. For an enzyme with a single catalytic site, Figure 2-24(a) shows how $d[P]/dt$, the rate of product production, depends on [S] when [E] is kept constant.

At low concentrations of S, the reaction rate is proportional to [S]; as [S] is increased the rate does not increase indefinitely in proportion to [S] but eventually reaches V_{max} at which it becomes independent of [S]. V_{max} is proportional to [E] and to a catalytic constant k_{cat} that is an intrinsic property of the individual enzyme; halving [E] reduces the rate at all values of [S] by one-half.

When interpreting curves such as those in Figure 2-24, bear in mind that all enzymatically catalyzed reactions include at least three steps: (1) the binding of the substrate (S) to the enzyme (E) to form an enzyme-substrate complex (ES); (2) the conversion of ES to the enzyme-product complex (EP); and (3) the release of the product (P) from EP, to yield free P:

$$E + S \xrightarrow{\text{binding}} ES \xrightarrow{\text{catalysis}} EP \xrightarrow{\text{release}} P + E$$

In the simplest case, the release of P is so rapid that we can write

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P$$

The reaction rate $d[P]/dt$ is proportional to the concentration of ES and to the catalytic constant k_{cat} for the given enzyme:

$$\frac{d[P]}{dt} = k_{cat}[ES] \quad (1)$$

To calculate [ES], we assume the reaction is in a steady state, so that k_1 [E] [S], the formation rate of [ES], is equal to the rate of its consumption, either by dissociation of uncatalyzed substrate at a rate of k_2 [ES] or by catalysis at a rate of k_{cat} [ES]:

$$k_1[E][S] = (k_2 + k_{cat})[ES] \quad (2)$$

If

$$[E]_{tot} = [E] + [ES] \quad (3)$$

(where $[E]_{tot}$ is the sum of the free and the complexed enzyme, or the total amount of enzyme), then we can combine equations (2) and (3) to obtain

$$[E]_{tot} = [E] + [ES] = \frac{(k_2 + k_{cat})}{k_1[S]}[ES] + [ES]$$

$$= [ES]\left[1 + \left(\frac{k_2 + k_{cat}}{k_1}\right)\left(\frac{1}{[S]}\right)\right]$$

If we define K_m, called the *Michaelis* constant, as

$$\frac{k_2 + k_{cat}}{k_1} \quad (4)$$

then

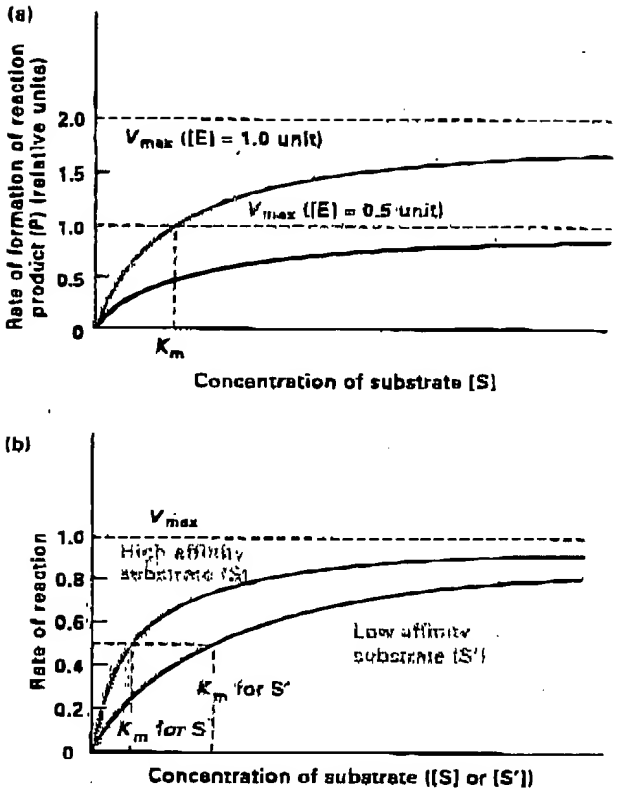
$$[ES] = \frac{[E]_{tot}}{1 + K_m/[S]}$$

Thus

$$\frac{d[P]}{dt} = k_{cat}[ES] = k_{cat}[E]_{tot}\frac{1}{1 + K_m/[S]}$$

$$= k_{cat}[E]_{tot}\frac{[S]}{[S] + K_m} \quad (5)$$

This equation fits the curves shown in Figure 2-24a. V_{max}, which is equal to $k_{cat}[E]_{tot}$, is the maximal rate of product formation if all recognition sites on the enzyme are filled with substrate. K_m is equivalent to the substrate concentration at which the reaction rate is half-maximal. (If $[S] = K_m$, then from equation (5) we calculate the rate of product formation to be $\frac{1}{2}k_{cat}[E]_{tot} = \frac{1}{2}V_{max}$.) For most enzymes, the slowest step is the catalysis of [ES] to [E] + [P]. In these cases, k_{cat} is much less than k_2, so that $K_m = (k_2 + k_{cat})/k_1 \cong k_2/k_1$ is equal to the equilibrium constant for binding S to E. Thus the parameter K_m describes the affinity of an enzyme for its substrate. The smaller the value of K_m, the more avidly the enzyme can bind the substrate from a dilute solution (Figure 2-24b) and the lower the value of [S] needed to reach half-maximal velocity. The concentrations of the various small molecules in a cell vary widely, as do the K_m values for the different enzymes that act on them. Generally, the intracellular concentration of a substrate is approximately the same as or greater than the K_m value of the enzyme to which it binds.

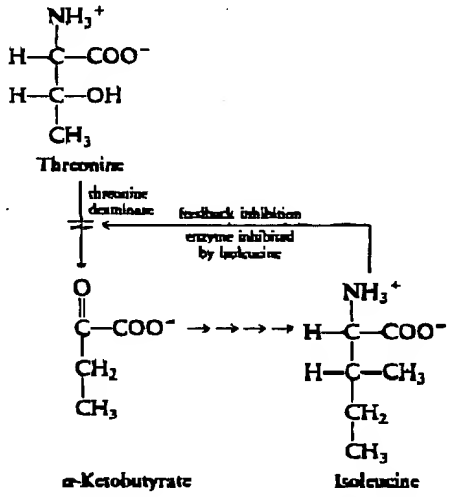


▲ **Figure 2-24** (a) The rate of a hypothetical enzymatically catalyzed reaction S → P for two different concentrations of enzyme [E] as a function of the concentration of substrate [S]. The substrate concentration that yields a half-maximal reaction rate is denoted by K_m. Doubling the amount of enzyme causes a proportional increase in the rate of the reaction, so that the maximal velocity V_{max} is doubled. The K_m, however, is unaltered. (b) The rates of reactions catalyzed by an enzyme with a substrate S, for which the enzyme has a high affinity, and with a substrate S', for which the enzyme has a low affinity. The V_{max} value is the same for S and S', but K_m is higher for S'.

The Actions of Most Enzymes Are Regulated

Many reactions in cells do not occur at a constant rate. Instead, the catalytic activity of the enzymes is *regulated* so that the amount of reaction product is just sufficient to meet the needs of the cell.

An Enzyme Can Be Feedback Inhibited in a Reaction Pathway Consider a series of reactions leading to the synthesis of the amino acid isoleucine, which is primarily used by cells as a monomer in the synthesis of proteins. The amount of isoleucine needed depends on the rate of protein synthesis in the cell. The first step in the synthesis of isoleucine is the elimination of an amino group, which converts the amino acid threonine to the compound α-ketobutyrate. Threonine deaminase—the enzyme that catalyzes this reaction—plays a key role in regulating the level of isoleucine. In addition to its substrate-binding sites for threonine, threonine deaminase contains a binding site for isoleucine. When isoleucine is bound there, the enzyme molecule undergoes a conformational change, so that it cannot function as efficiently. Thus isoleucine acts as an *inhibitor* of the reaction for the conversion of threonine. If the isoleucine concentration in the cell is high, the binding of isoleucine to the enzyme temporarily reduces the rate of isoleucine synthesis:



This is an example of *feedback inhibition*, whereby an enzyme that catalyzes one of a series of reactions is inhibited by the ultimate product of the pathway.

In isoleucine synthesis, as in most cases of feedback inhibition, the final product in the reaction pathway inhibits the enzyme that catalyzes the first step that does not also lead to other products. The suppression of enzyme function is not permanent. If the concentration of free isoleucine is lowered, bound isoleucine dissociates from the enzyme, which then reverts to its active conformation. The binding of the inhibitor isoleucine to the enzyme and its subsequent release can be described by the equilibrium-binding constant K_i, which is similar to the constant K_m used for substrate binding:

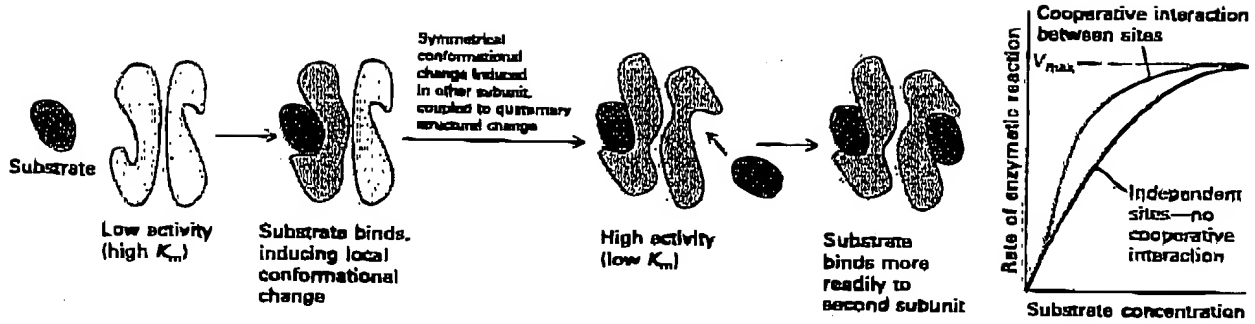
$$[E \cdot Ile]_{inactive} \rightleftharpoons [Ile] + [E]_{active}$$

$$K_i = \frac{[Ile][E]_{active}}{[E \cdot Ile]_{inactive}}$$

Many Enzymes Have Multiple Binding Sites for Regulatory Molecules Some enzymes have binding sites for small molecules that affect their catalytic activity; a stimulator molecule is called an *activator*. Enzymes may even have multiple sites for recognizing more than one activator or inhibitor. In a sense, enzymes are like microcomputers; they can detect concentrations of a variety of molecules and use that information to vary their own activities. Molecules that bind to enzymes and increase or decrease their activities are called *effectors*. Effectors can modify enzymatic activity because enzymes can assume both active and inactive conformations: activators are positive effectors; inhibitors are negative effectors. Effectors bind at *regulatory sites*, or *allosteric sites* (from the Greek for "another shape"), a term used to emphasize that the regulatory site is an element of the enzyme distinct from the catalytic site and to differentiate this form of regulation from competition between substrates and inhibitors at the catalytic site.

Multimeric Organization Permits Cooperative Interactions among Subunits Many enzymes and some other proteins are multimeric—that is, they contain several copies, or subunits, of one or more distinct polypeptide chains. Some multimeric enzymes contain identical subunits, each of which has a catalytic site and possibly an effector site. In other enzymes, regulatory sites and catalytic sites are located on different subunits, each with a particular structure. On binding an activator, inhibitor, or substrate, a subunit undergoes a conformational change, usually small, that triggers a change in quaternary structure. This quaternary rearrangement favors a similar conformational change in the other subunits, thereby increasing their affinity for the type of ligand initially bound (Figure 2-25). When several subunits interact cooperatively, a given increase or decrease in substrate or effector concentration causes a larger change in the rate of an enzymatic reaction than would occur if the subunits acted independently. Because of such *cooperative interactions*, a small change in the concentration of an effector or substrate can lead to large changes in catalytic activity.

Cooperative interactions among the four subunits in hemoglobin demonstrate clearly the advantages of multimeric organization. The binding of an O_2 molecule to any one of the four chains (each hemoglobin chain binds one O_2) induces a local conformational change in that subunit. This change can in turn induce a large change in quaternary structure. The quaternary change involves a rearrangement of the positions of the two α and two β chains in the tetramer. The local conformational changes that accompany O_2 binding can then occur more readily in the remaining subunits, increasing their affinity for oxygen. The binding of a second O_2 makes the quaternary structural change even more likely. The cooperative



▲ **Figure 2-25** A cooperative interaction between active sites (two identical subunits of a hypothetical enzyme). The binding of a substrate to one subunit of a multimeric enzyme induces a conformational change in the adjacent subunit, which lowers the K_m for the binding of the substrate there. Thus a small change in the substrate concentration can cause a much larger increase in the reaction rate than would occur if there were no cooperative interactions between active sites.

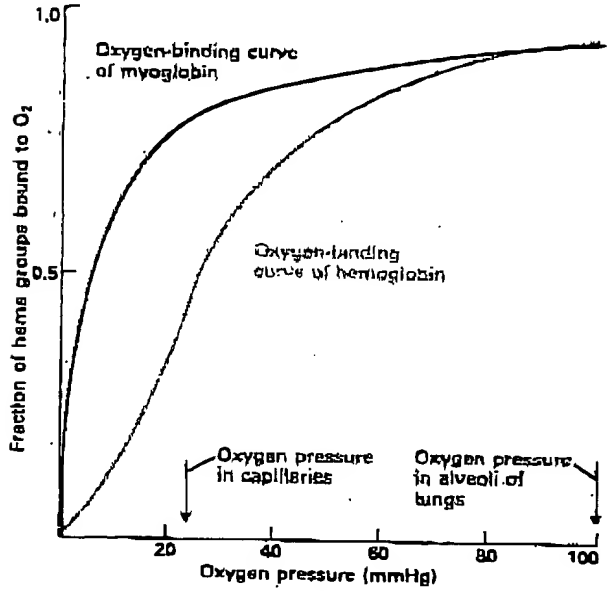
interaction between the chains causes the molecule to take up or lose four O_2 molecules over a much narrower range of oxygen pressures than it would otherwise. As a result, hemoglobin is almost completely oxygenated at the oxygen pressure in the lungs and largely deoxygenated at the oxygen pressure in the tissue capillaries (Figure 2-26).

The contrast between hemoglobin and myoglobin is revealing. Myoglobin is a single-chain oxygen-binding protein found in muscle. The oxygen-binding curve of myoglobin has the characteristics of a simple equilibrium reaction:

$$E + O_2 \rightleftharpoons E{-}K_{O_2}$$

Myoglobin has a greater binding affinity for O_2 (a lower K_{O_2}) than hemoglobin at all oxygen pressures. Thus, at the oxygen pressure in capillaries, O_2 moves from hemoglobin into the muscle cells, where it binds to myoglobin, ensuring the efficient transfer of O_2 from blood to tissues.

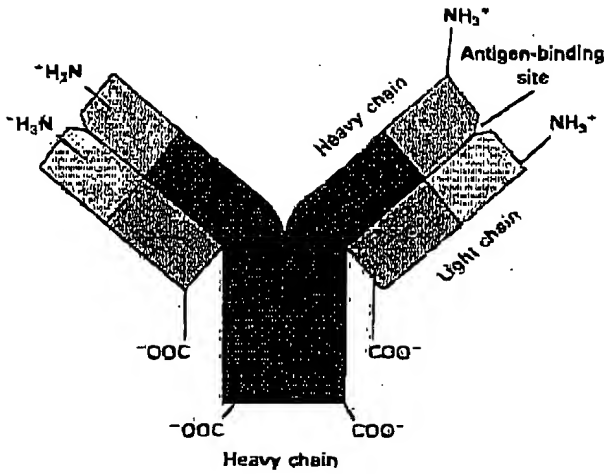
The quaternary-structure rearrangements associated with multimeric organization also provide a way for the effects of activator or inhibitor binding at an allosteric site to be transmitted to a distant catalytic site without large changes in the secondary or tertiary structure of an enzyme, which would be incompatible with the principle that a particular primary structure must adopt a unique folded conformation. Thus, for example, small conformational changes in a domain in response to binding of an effector molecule would produce a quaternary-structure change, which amplifies the conformational signal and allows it to be transmitted robustly to other parts of the enzyme, where it would induce a small conformational change affecting enzymatic activity. Membrane-embedded receptor proteins that must transmit a conformational signal from one side of a membrane to the other are also likely to be multimeric; they transmit the signal by quaternary-structure rearrangement or by an effector-induced shift in the monomer-multimer equilibrium.



◀ **Figure 2-26** The binding of oxygen to hemoglobin depends on cooperative interactions between the four chains. The graph shows the fraction of heme groups in hemoglobin and in myoglobin bound to O_2 as a function of the oxygen pressure. Note that the binding activity of hemoglobin increases sharply over a narrow range of oxygen pressures (20–40 mmHg). Hemoglobin is saturated with O_2 in the lungs, but it releases much of its bound O_2 at the low oxygen pressure in the tissue capillaries. At any oxygen pressure, myoglobin has a higher affinity for O_2 than hemoglobin does. As myoglobin is a principal muscle protein, this property allows oxygen to be transferred from blood to muscle.

Enzymes Are Regulated in Many Ways The activities of enzymes are extensively regulated so that the numerous enzymes in a cell work together harmoniously. All metabolic pathways are closely controlled at all times. Synthetic reactions occur when the products of these reactions are needed; degradative reactions occur when molecules must be broken down. Kinetic controls affecting the activities of key enzymes determine which pathways are going to be used and the rates at which they will function.

Regulation of cellular processes involves more than simply turning enzymes on and off, however. Some regulation is accomplished through *compartmentation*. Many enzymes are localized in specific compartments of the cell, such as the mitochondria or lysosomes, thereby restricting the substrates, effectors, and other enzymes with which an enzyme can interact. In addition, compartmentation permits reactions that might otherwise compete with one another in the same solution to occur simultaneously in different parts of a cell. Cellular processes are also regulated through the control of the rates of enzyme synthesis and destruction.



▲ **Figure 2-27** The structure of an antibody molecule illustrated in an immunoglobulin (IgG) made of four polypeptide chains: two identical heavy chains (blue) and two identical light chains (orange). Each antigen-binding site is formed by the N-terminal segments of a heavy and a light chain. The N-termini are highly variable in sequence, giving rise to the wide range of antibody specificity.

Antibodies

Enzymes are not the only proteins that bind tightly and specifically to smaller compounds. The insulin receptor on the surface of a liver cell, for example, can bind to insulin so tightly that the receptors on a cell are half-saturated when the insulin concentration is only 10^{-9} *M*. This protein does not bind to most other compounds present in blood; it mediates the specific actions of insulin on liver cells. A molecule other than an enzyme substrate that can bind specifically to a macromolecule is often called a *ligand* of that macromolecule.

The capacity of proteins to distinguish among different molecules is developed even more highly in blood proteins called *antibodies*, or *immunoglobulins*, than in enzymes. Animals produce antibodies in response to the invasion of an infectious agent, such as a bacterium or a virus. Antibodies will be discussed at length in Chapter 25. We introduce them here because they will appear as critical reagents in the discussions of many intervening chapters.

The recognition site of an antibody can bind tightly to very specific sites—generally on proteins or carbohydrates—on the surface of the infectious agent. Experimentally, animals produce antibodies in response to the injection of almost any foreign polymer; such antibodies bind specifically and tightly to the invading substance but, like enzymes, do not bind to dissimilar molecules. The antibody acts as a signal for the elimination of infectious agents. When it binds to a bacterium, virus, or virus-infected cell, certain white blood cells (leucocytes) recognize the invading body as foreign and respond by destroying it. The specificity of antibodies is exquisite: they can distinguish between proteins that differ by only a single amino acid and between the cells of different individual members of the same species.

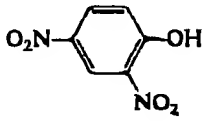
All vertebrates can produce a large variety of antibodies, including ones that bind to chemically synthesized molecules. Exposure to an antibody-producing agent, called an *antigen*, causes an organism to make a large quantity of different antibody proteins, each of which may bind to a slightly different region of the antigen. For a given antigen, these constellations of antibodies may differ from one member of a species to another.

Antibodies are formed from two types of polypeptides: heavy chains, each of which is folded into four domains, and light chains, each of which is folded into two domains (Figure 2-27). The N-terminal domains of both heavy and light chains are highly variable in sequence, giving rise to the specific binding characteristics of antibodies.

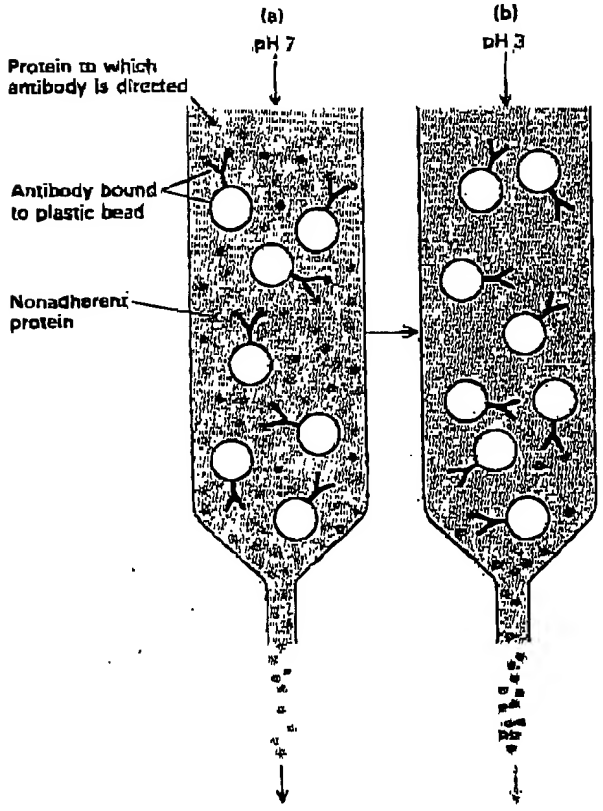
Antibodies Can Distinguish among Closely Similar Molecules

The sequence of bovine insulin is identical to that of human insulin, except at three amino acids. Yet when bovine insulin is injected into people, some individuals respond by synthesizing antibodies that specifically recognize the specific amino acids in the bovine molecule, even though human beings generally do not produce anti-

bodies that recognize their own insulin. Injecting mouse albumin—the major serum protein—into mice does not elicit the production of antialbumin antibodies. However, if a small molecule, such as 2,4-dinitrophenol (DNP)



is coupled to the albumin, mice do produce antibodies that bind specifically to the modified region of the protein—in this case, to the dinitrophenyl group. A small group capable of eliciting antibody production is called a *hapten*. The anti-DNP/albumin antibody will not bind to albumin that is not complexed with DNP or that is modified by other haptens (even phenyl groups with different substituents).



▲ **Figure 2-28** The purification of a protein from a mixture by affinity chromatography. (a) In step 1, the mixture is filtered through a column containing antibody molecules that are specific for the desired protein. Only that protein binds to the antibody matrix; any other proteins in the mixture are eluted. (b) In step 2, a solution such as acetic acid is added to disrupt the antigen-antibody complex, so that a pure protein is eluted from the column.

Antibodies Are Valuable Tools for Identifying and Purifying Proteins

Because they bind so selectively to proteins, antibodies can be used experimentally to isolate one protein from a complex mixture. In one technique, *affinity chromatography*, a pure antibody is chemically coupled to tiny plastic beads, which are then placed in a small column. When a protein solution is applied, only the protein to which the antibody is directed adheres to the column; all nonadherent proteins pass through the column unimpeded (Figure 2-28a). The adherent protein can then be eluted by adding a solution that disrupts the binding between the protein and the antibody (Figure 2-28b). Similarly, antibodies can be used to detect specific proteins in cells or other biological materials.

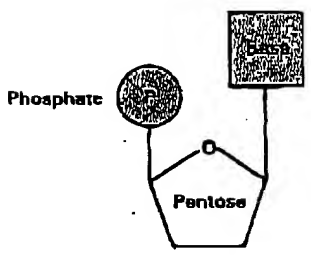
Nucleic Acids

Cells receive instructions about which proteins to synthesize and in what quantities from *nucleic acids*—the molecules that store and transmit information in cells. As in many systems of communication, this information is processed in the form of a code. The translation of this code is described in Chapter 3. Here, we examine the chemical structures of the molecules that store the encoded information.

Nucleic Acids Are Linear Polymers of Nucleotides Connected by Phosphodiester Bonds

Cells have two closely related information-carrying molecules: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Like proteins, DNA and RNA are linear polymers. However, the number of monomers in a nucleic acid is generally much greater than the number of amino acids in a protein. Cellular RNAs range in length from tens to thousands of units. The number of units in a DNA molecule can be in the millions.

DNA and RNA each consist of only four different monomers, called *nucleotides*. A nucleotide has three parts: a phosphate group, a *pentose* (a five-carbon sugar molecule), and an organic *base* (Figure 2-29). In RNA,



◀ **Figure 2-29** A schematic diagram of the structure of a nucleotide.